Preliminary Studies on the Chemical Nature of Mosquito-Breeding Waters in Delhi *

by S. S. SEHGAL [1] & M. K. K. PILLAI [2]

For mosquito-control programmes a good knowledge of the biology and ecology of mosquitos is of paramount importance. Mosquito breeding generally occurs in a wide range of habitats with different types of waters. These are known to be specific for many mosquito species, although the factors involved are not clearly known. The physical and chemical nature of the water probably determines the selection of breeding sites. Peterson & Rees (1966) in their study of surface waters have shown that *Aedes dorsalis* (Meigen) prefers soil with a high salinity for oviposition and breeding whereas *Aedes nigromaculis* (Ludlow) chooses to breed in less saline soil. Smith (1966) reported that three species of *Culicoides* bred in soils with a slightly acid pH range. From an extensive study of chemical factors in waters of the tree-hole-breeding mosquitos, Peterson & Chapman (1969) have shown a definite relationship between the chemical constitution of the habitats and the 13 species of *Culicoides* inhabiting them.

Pillai et al. (1968), in a survey of the breeding sites of *Aedes* in Delhi during September–October 1967, have shown a high frequency of three species, *Aedes albopictus* (Skuse), *Aedes aegypti* (L.) and *Aedes vittatus* (Bigot). Abundance of mosquitos in Delhi always follows rainfall, which usually occurs from July to August. These three species of *Aedes* were to be found either alone or together in certain waters and sometimes even together with species of *Anopheles* and *Culex*. With a view to relating the physicochemical factors of the waters to the occurrence of particular species (alone or together), a preliminary study was conducted during the period July–October 1969. The study was primarily concerned with the different species of *Aedes* because of their medical importance as vectors of haemorrhagic fever in various parts of India (Rao, 1967). However, the genera *Anopheles* and *Culex* were also included in the study as their ecological niches overlapped those of *Aedes*.

Materials and methods

Water samples for chemical analysis were collected from a few localities in Delhi which had been found positive for mosquito breeding in our earlier survey (Pillai et al., 1968). During the present studies, Delhi had a total rainfall of 445.7 mm, and the mean atmospheric temperature was in the range from 24.8°C to 34.0°C. The samples were taken from temporary water collections formed by rain. The water temperature was measured before the samples were collected. The samples were analysed for turbidity, dissolved oxygen, total alkalinity and ions such as bicarbonate, chloride, nitrate, phosphate and sulfate by means of a portable, field-model Hach DR-EL water-engineer's laboratory kit. Mosquito eggs, larvae and pupae were collected in a close-mesh strainer. After being counted, they were allowed to develop in the laboratory in the same water in order that proper identifications could be made from the adults. Identification to species was made only in the case of *Aedes*.

Results

In all, 46 water samples were collected from breeding sites during the period of the study and 32 of the sites were positive for mosquito breeding; only these were investigated in detail. Nine sites carried a mixed population of 2 or more species. The rest, which carried only a single species, consisted of 12 sites with *Anopheles*, 4 with *Culex*, 3 with *Ae. vittatus*, 2 with *Ae. albopictus* and 2 with *Ae. aegypti*. The water temperature of the sites examined was in the range 29°C–35°C. Bicarbonate ions were almost negligible in all the samples analysed.

The table summarizes the chemical composition of the sites in relation to the various species of mosquitos inhabiting them. *Anopheles* and *Culex* were found to breed in highly turbid waters, in contrast to the three species of *Aedes*, which inhabited waters of low turbidity. Breeding waters of *Aedes*

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CHEMICAL CHARACTERISTICS OF WATERS FROM MOSQUITO-BREEDING SITES

Mosquito [a]	Turbidity (Jackson turbidity units)	Dissolved oxygen (ppm)	pH	Total alkalinity (ppm)	Chloride (ppm)	Sulfate (ppm)	Nitrate (ppm)	Phosphate (ppm)
Anopheles alone								
Range	15–300	1–6	7.6–8.7	160–830	25–450	6–117	10–16	0.8–6.0
Average	179	3.5	8.3	314	173	43	12.2	3.30
Culex alone								
Range	70–280	1–4	7.6–8.0	190–380	20–100	23–50	10–13	1.1–30
Average	150	2.6	7.8	267	57	41	11.6	16.60
Ae. vittatus & *Culex* [1:10]								
Range	10–85	1–7	8.2–9.4	75–220	30–100	22–60	9–11	0.5–7.0
Average	48.7	3.7	8.5	151	69	49	10.2	2.83
Ae. vittatus & *Culex* & *Anopheles* [1.8:2:1]								
Range	50–60	3–7	8.2–8.7	130–200	40–65	7–10	7–14	4.4–10.5
Average	53.3	4.6	8.3	160	50	8	11.0	7.60
Ae. vittatus alone								
Range	18–50	4–11	8.8–10	80–95	25–65	42–100	8–15	0.4–2.0
Average	29.3	8.3	9.3	87	42	72	12.3	0.63
Ae. albopictus alone								
Range	50–75	3–6	7.6–7.6	55–150	20–50	55–100	10–13	0.4–2.0
Average	62.5	4.5	7.6	103	35	76	11.5	1.20
Ae. aegypti alone								
Range	25–70	5–5	7.5–7.6	170–180	47–50	55–85	8–12	0.9–1.0
Average	47.5	5.0	7.5	175	49	70	10.0	0.95
Ae. aegypti & *Culex* [0.8:1]								
Range	—	—	—	—	—	—	—	—
Average	30	5.0	7.7	145	55	60	11.0	0.7

[a] The figures in brackets indicate the proportion of different mosquito species found in the sites carrying more than one species.

showed a higher oxygen content than those of *Culex* and *Anopheles*. Among the *Aedes* species, *Ae. vittatus* was restricted to waters of high oxygen content and low turbidity. In the waters with mixed populations of the different *Aedes* species, a proportionate variation in relative numbers by species was observed related to turbidity and oxygen content.

Ae. vittatus always preferred an alkaline pH, in the range 8.8–10. The distribution of *Ae. aegypti* and *Ae. albopictus* was almost identical; they preferred a slightly alkaline pH of about 7.5. *Anopheles* and *Culex* breeding waters had pH values between 7.5 and 8.5.

Total alkalinity was least in the breeding waters

of *Ae. vittatus*, the range being 80 ppm–95 ppm. *Ae. aegypti* favoured waters with alkalinity values of 170 ppm–180 ppm, whereas *Ae. albopictus* showed an intermediate preference of 55 ppm–150 ppm. *Culex* and *Anopheles* preferred very high alkalinity.

The chloride content was high in breeding waters of *Anopheles* and low in those of *Culex*. The habitats of the three species of *Aedes* did not show much variation in chloride content.

The sulfate ions in water samples showed considerable variation. There was an average sulfate content of 70 ppm or a little more in the *Aedes* habitats, but a low sulfate content in the breeding waters of *Anopheles* and *Culex*.

The nitrate content was almost the same in all the samples. However, the phosphate content was variable: 16.6 ppm in *Culex* samples and 3.3 ppm in *Anopheles* samples; the breeding of *Aedes* species was restricted to waters of very low phosphate content.

Discussion

The ecological parameters such as pH, turbidity, dissolved oxygen and alkalinity, and chloride, sulfate and phosphate content observed in the present investigation clearly indicate that mosquitos exhibit a certain species-specificity with regard to the chemical nature of the environment (habitat). The data obtained from sites inhabited by more than one species of mosquito also showed a distinct trend when the relative numbers of the different species are taken into account. Bast (1964), from his studies on the chemical nature of pond waters, has suggested that specific conductance and acidity may be taken as criteria in ecologically defining the difference between mosquito-producing and non-producing areas. Studies on the chemical nature of the waters of tree-hole-breeding mosquitos have indicated a definite relationship between the chemical characteristics and the 13 *Culicoides* species which selected them (Peterson & Chapman, 1969).

The present study clearly brings out the relationship between the chemical nature of breeding waters and species distribution. The *Aedes* species examined were restricted to less turbid waters with a high dissolved-oxygen content, and *Ae. vittatus* is shown to be more demanding than the others in this respect. *Anopheles* and *Culex*, on the other hand, prefer more turbid waters with a much lower oxygen content. Another parameter which influences species-specificity is the pH; here again *Ae. vittatus* prefers a highly alkaline pH and the others a slightly or moderately alkaline pH. Peterson & Chapman (1969) found 13 species of tree-hole-breeding *Culicoides* inhabiting waters with a pH range of 6.5–8.5. Among those breeding in surface waters, *Ae. dorsalis* was found to prefer waters with a pH of 6.0–10.1, while *Ae. nigromaculis* preferred a pH of 4.7–9.2 for oviposition and breeding (Chapman, 1960). Laboratory studies on the ovipositional responses of *Ae. aegypti* showed their preference for waters with a pH range of 6.0–9.2 (Pillai & Madhukar, 1969).

Anopheles and *Culex* habitats, in contrast to those of *Aedes*, had a high total alkalinity, with the difference that *Anopheles* preferred waters with a higher chloride content. Chapman (1960) reported the breeding of *Ae. dorsalis* in waters with a chloride range of from 14 ppm to 42 778 ppm and of *Ae. nigromaculis* in waters with 18 ppm–391 ppm chloride. Peterson & Rees (1967) have found that *Ae. dorsalis* preferred higher concentrations of sodium chloride and calcium chloride while *Ae. nigromaculis* was more selective for lower concentrations of these salts, as indicated by the numbers of eggs laid and by the egg hatch. The present data, however, did not show any notable difference in chloride content between the habitats of the three *Aedes* species studied. The phosphate content was generally low in all the sites examined.

In conclusion, it may be stated that the physical and chemical nature of the habitat seems to determine the species-specificity when the adult mosquitos select the breeding sites. Thus the waters in which *Ae. vittatus* bred were characterized by low turbidity, high oxygen content, a highly basic pH and a low phosphate content; and the habitats of *Ae. albopictus* and *Ae. aegypti* were almost identical in turbidity, oxygen content, pH, alkalinity, chloride and phosphate ions. However, in the present study they were not found together in the same waters. It is known that *Ae. aegypti* prefers urban areas with human habitations while *Ae. albopictus* selects suburban and rural areas for breeding. This may be due to the anthropophilic nature of *Ae. aegypti* (Macdonald, 1956). The present findings suggest that the ecological niches present in the urban areas are suitable for *Ae. albopictus*, and thus there may be a possibility of this species extending its distribution into the urban areas if *Ae. aegypti* is eradicated, as has been suggested by Gilotra et al. (1967) in studies on the possible competitive displacement of the one species by the other in Calcutta. Although individually the

breeding habitats of *Culex* and *Anopheles* were chemically distinct, in a few cases these mosquitos were also found to breed along with *Aedes* species where the chemical make-up of the water was somewhat different. However it is not certain whether these chemical differences alone are responsible for differences in species breeding (Peterson & Chapman, 1969). The differences observed may be partly due to the amount of mosquito breeding which might have taken place previously in the sites sampled, although whether this influences the ovipositional preference of the mosquitos has yet to be proved conclusively.

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The Blood Incubation Infectivity Test: a Simple Test which may Serve to Distinguish *Trypanosoma brucei* from *T. rhodesiense* *

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For the last 60 years or more, one of the great obstacles to the study of the epidemiology and epizootiology of African human trypanosomiasis has been the difficulty of distinguishing *Trypanosoma brucei*, which is by definition not infective to man, from the human-infective *T. rhodesiense*. So far, the only means of making this distinction has been a direct test of infectivity in a human volunteer, a test which, for obvious reasons, can only rarely be performed.

It follows that if a strain of trypanosomes of the *T. brucei* subgroup is isolated from tsetse or domestic or game animals there is no means of knowing whether it is truly *T. brucei* or the human-infective *T. rhodesiense* unless it has been tested for infectivity to man. Because of this limitation there are relatively few such strains, isolates which have been proven to be non-infective to man, and these are commonly distinguished by the name *T. brucei* (*sensu stricto*). One serious consequence of the lack of a convenient test to distinguish *T. brucei* (*sensu stricto*) from *T. rhodesiense* has been the impossibility of investigating the relative frequency and distribution of these species in tsetse or potential reservoir animals.

We have recently developed a test (Blood Incubation Infectivity Test) which, with the limited number of strains available to us, has been consistently successful in making this distinction. When rats or mice, inoculated with the strain of trypanosome to be tested, become positive as assessed by microscopical examination, cardiac blood is withdrawn aseptically and 0.25 ml added to each of two Bijou bottles. The first bottle, representing the test

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